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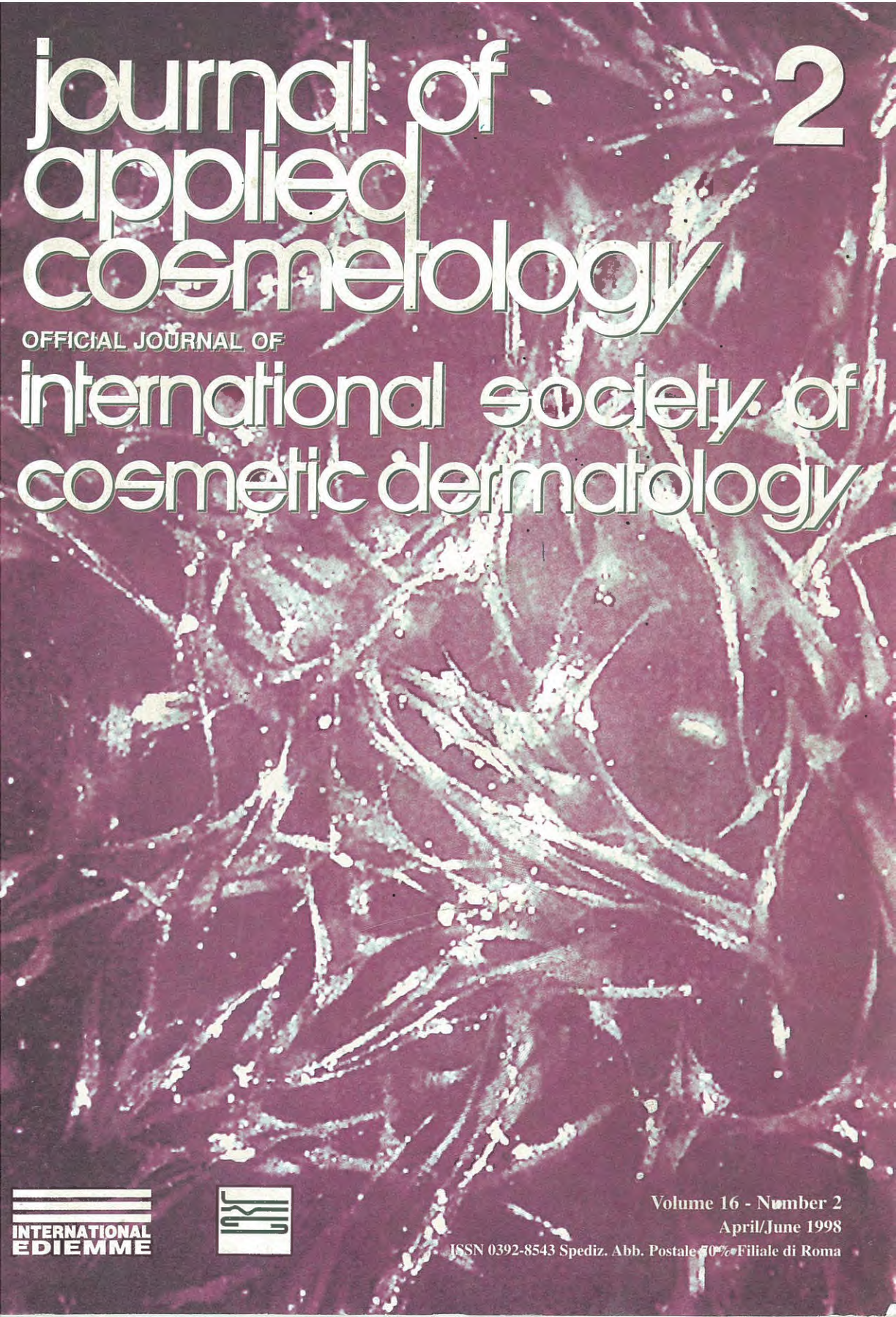

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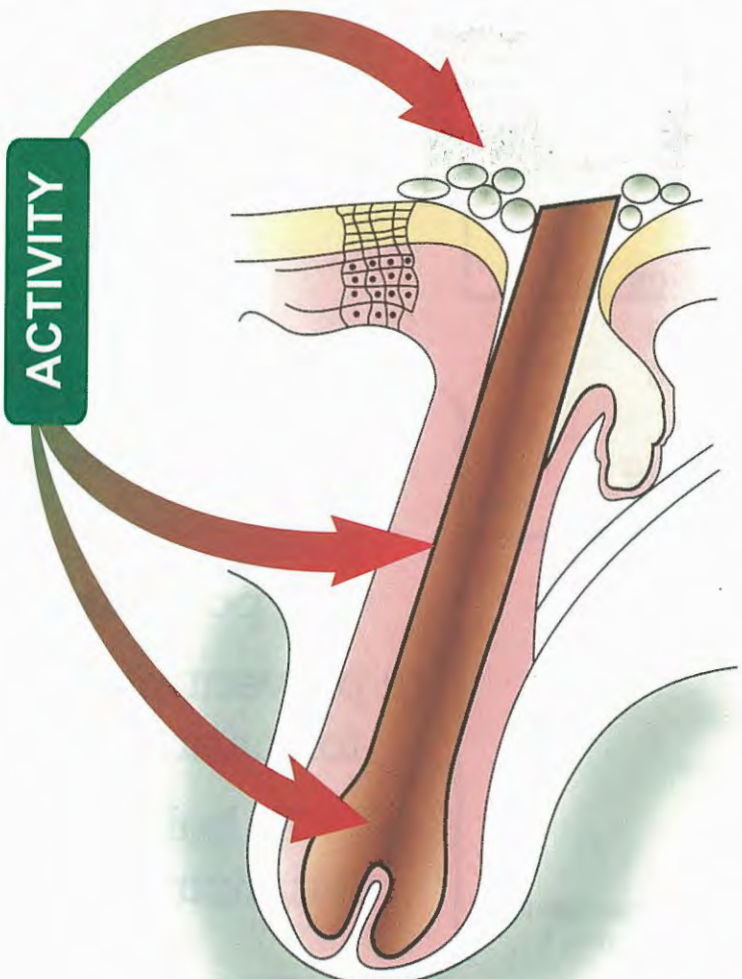

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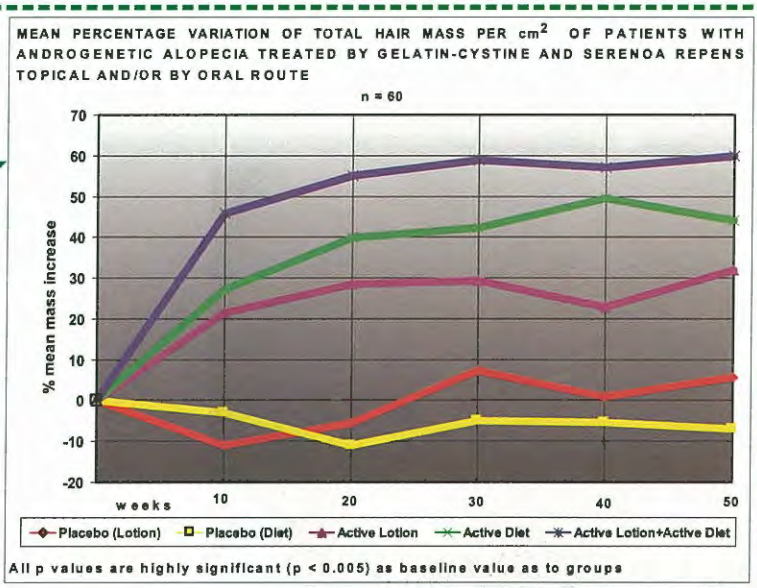

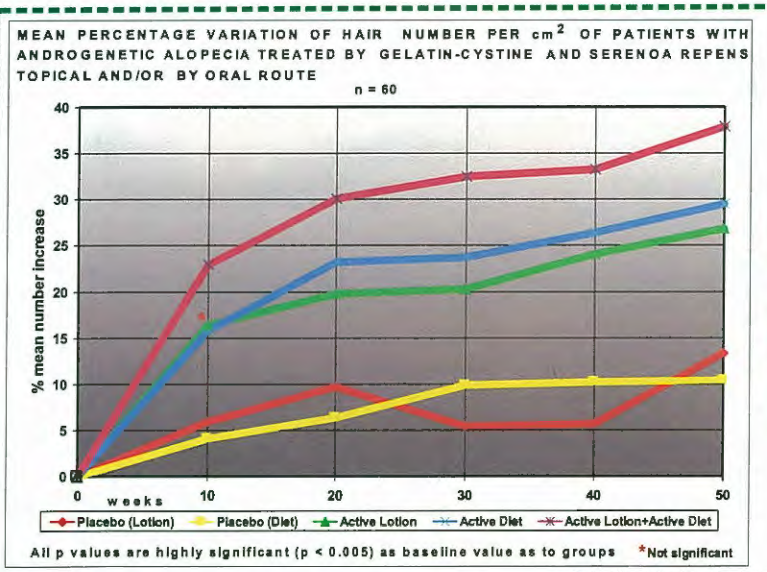

38%

increase in hair number

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1) Morganti P., Fabrizi G., James B. And Bruno C. *J. Appl. Cosmetol.***16**,57,1998

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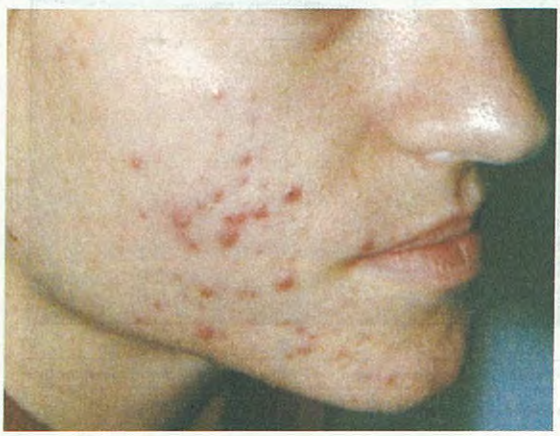

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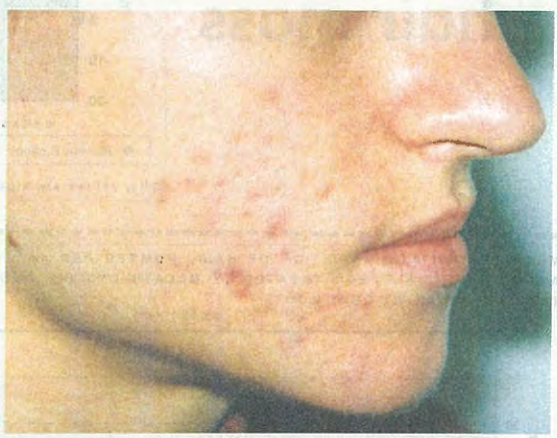

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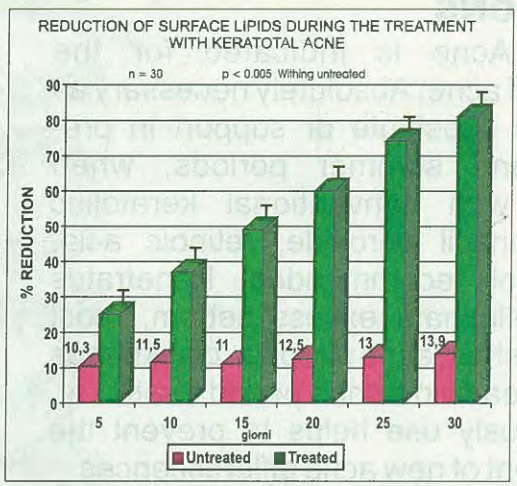

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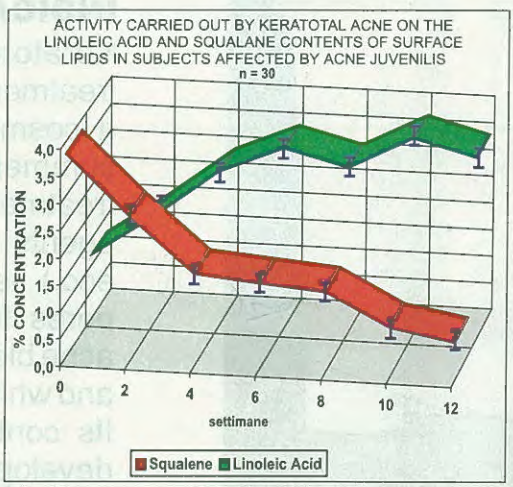
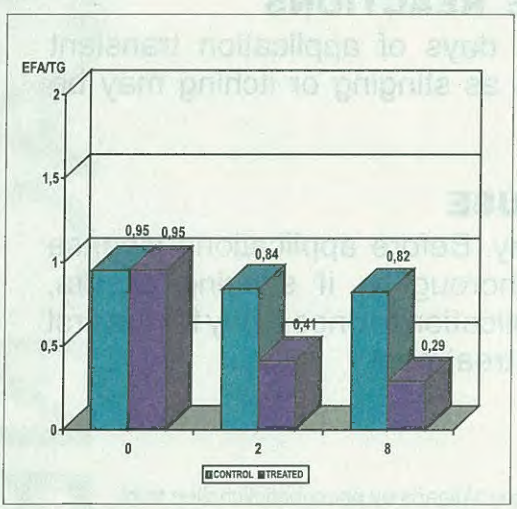


⇒ *Decreases the Squalene content of acne affected skin*



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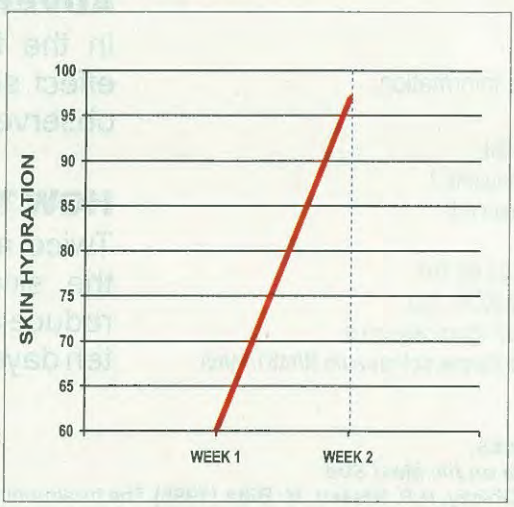

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DESCRIPTION

Keratotal Acne is a special fat-free lamellar phosphatidylcholine emulsion developed for the treatment of acne. It is delivered in a special phospholipidic-vehicle linoleic acid rich which contains glicolic acid and salicilic acid partially neutralized by a special patented blend of aminoacids

INDICATIONS

Keratotal Acne is indicated for the treatment of acne. Absolutely necessary as a cosmetic substitute or support in pre-summer and summer periods, when treatment with conventional keratolitic agents (benzoil peroxide, retinoic acid, ecc.) is not recommended. Penetrates pores to eliminate excess sebum, most acne blemishes, acne pimples, blackheads and whiteheads in a short period treatment. Its continously use helps to prevent the development of new acne efflorescences

ADVERSE REACTIONS

In the first days of application transient effect such as stinging or itching may be observed

HOW TO USE

Twice a day. Before applications cleanse the skin thoroughly; if stinging occurs, reduce application to once a day for the first ten days of treatment

REFERENCES:
*1,2 - **Data on file Mavi Sud***
3 - **M. Ghiczy, H.P. Nissen, H. Biltz (1996)** The treatment of Acne Vulgaris by phosphatidilcholine from Soybeans, with a high content of linoleic acid. *J. Appl. Cosmetol.* **14,** 137-145

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Trimestrale di Dermatologia Cosmetologica
Quarterly Review of Cosmetic Dermatology

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The **JOURNAL OF APPLIED COSMETOLOGY** is an international journal devoted to publisching original papers, reviews and other material which represent a useful contribution to research on the skin and on cosmetics.
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The challenges and the chances
Paris 20-22 April, 1999

4th Scientific Conference of ASCS
Cosmetics, safety and efficacy: dream and fact
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J. Appl. Cosmetol. 16, 27-36 (April-June 1998)

EFFECTS OF UVR ON IMMUNE RESPONSE OF SKIN AND EVALUATION OF SUNSCREEN

Zhou Hua*, Zhu Huigang*, Zhong Zhennong.**
*Department of Environmental Health, Shanghai Medical University
**Shangai Epidemic Prevention Station

Received: *December 10, 1997*

Key words: *Ultraviolet Radiation, Sunscreens, Epidemiology, Immunization*

Synopsis

1. It is an important work to monitor the ground level UVR, especially for a long time. The highest means UVR irradiate value at noon is observed in July (UV-420, 6814 μW/cm^2; UV-365, 31 34 μW/cm^2) or August (UV-297, 31.5 μW/cm^2) at shanghai. The trend showed that radiation power of summer > that of autumn > that of spring > that of winter. The highest means UVR of a day appeared around noon. The UVA is nearly 100 times higher than UVB.
2. Sun protection factor (SPF) is an important index to evaluate the effectiveness of sunscreen. It is urgent to establish the standards on evaluating the efficacy of sunscreen in our country. We have set up in vivo and in vitro methods to detect the SPF of sunscreen.
3. Though majority of people realized the harmful effects of UVR, they neglected to protect themselves from exposure to UVR. It is urgent to take some propaganda against UVR.
4. UVA radation has the capacity to damage collagen I and collagen III. It is believed that UVA plays a important role in the induction of photodamage of the skin,
5. UVR can suppress immune responses of the skin. Cis-urocanic acid (cis-UCA) has been suggested as a photoreceptor for UV and has been demonstrated to suppress immune responses in delayed type hypersensitivity (DTH) in mice.

Riassunto

1. È un importante lavoro monitorare gli UVR a livello del suolo, soprattutto per un lungo periodo di tempo. Il valore più alto di irradiazione degli UVR a mezzogiorno si rileva a luglio (UV-420, 6814 μW/cm^2; UV-365, 3184 μW/cm^2) o agosto (UV-297, 31.5 μW/cm^2) a Shangai. La tendenza ha mostrato il potere di irradiazione dell'estate > di quello dell'autunno > di quello della primavera > di quello dell'inverno. I più alti valori degli UVR in una giornata sono risultati essere quelli di mezzogiorno. Gli UVA sono circa 100 volte più alti degli UVB.
2. Il fattore di protezione dal sole (SPF=Sun Protection Factor) è un indice importante per valutare l'efficacia di uno schermo solare. È urgente stabilire gli standard per la valutazione degli schermi solari nel nostro paese. Noi abbiamo messo a punto metodi in vivo ed in vitro per rilevare il SPF degli schermi sol

3. Sebbene la maggior parte degli individui siano al corrente degli effetti nocivi degli UVR essi trascur di proteggersi dall'esposizione agli UVR. È urgente iniziare delle campagne di informazione di ma sugli UVR.
4. Le radiazioni UVA hanno la capacità di danneggiare il collagene I ed il collagene III. Si ritiene c gli UVA giochino un ruolo importante nel fotodanneggiamento cutaneo.
5. Gli UVR possono annullare le risposte immunitarie della pelle. È stato indicato nell'acido cis-urocanic (cis-UCA) un fotorecettore per gli UV ed è stato dimostrato che esso annulla le risposte immunitari nella DTH (delayed type hypersensitivity) dei topi.

INTRODUCTION

Ultraviolet (UV) is one of the non-ionizing radiaton in the electromagnetic spectrum and lies within the range of wavelength 100 nm to 400 nm. The short wavelength limit of the UV region is often taken as the boundary, between the ionizing radiation spectrum (<100 nm) and the non-ionizing radiation spectrum. UV can be classified into UVA (315-400 mn), UVB (280-315 nm) and UVC (100-280 nm) regions, although other conventions for UVA, UVB and UVC wavelengths bands are in use.

Exposure to UV occurs from both natural and artificial sources. The sun is the principal source of exposure for most people. In some special situation, exposure may come from artificial sources which include various lamps used in medicine, industry, commerce, research and the home.

Short exposure to UVR may be beneficial for health as the exposed skin can generate vitamin D_3. There are also deleterious effects on human health. The direct hazards are confined to the skin and the eyes because of the limited penetration of UVR into biological tissue. Excessive exposure can give rise to skin burns and blistering, which cause severe discomfort and systemic upset. Acute exposure may damage the cornea and conjunctiva, for example causing inflammation of the eye as the result of exposure to ambient UVR from surface such as snow with heightened reflectivity. Chronic exposure may also affect the skin by increasing aging effects and the risk of cancer and probably increases the risk of certain types of cataracts in the eyes. Small exposure to UVR can affect the skin's immune system and may enhance the risk of infection and decrease the effectiveness of vaccines in humans.

Globe environmental pollution becomes a more and more serious problem as industrialization being speeded up. With depletion of the stratospheric ozone people and the environment will be exposure to higher intensities of UV. The sequences of this added UV exposure are considered so serious that it was a major topic for discussion at the World Environment Conference, held in Rio de Janeiro in 1992. In Agenda 21, adopted by the Conference, it was specifically recommended to "undertake, as a matter of urgency, research on the effects on human health of increasing ultraviolet radiation reaching the earth's surface as the consequence of depletion of the stratospheric ozone layer".

The increasing UV radiation will be a disaster to all livng things including man. What shall we do? How can we prevent such unfortunate? As one of WHO Global Strategy for Health and Environment, a monograph has been drafted to provide the essential authoritative review on which future research programs in UV can progress. In recent years, researches on relationship between human and UVR have been taken into account in abroad. In contrast, there aro few studies having carried out at home. As a environmental researcher, we should do out best to protect our living environment.

Part 1. SOLAR RADIATION MEASUREMENTS

The sun is the main source of ultraviolet radiation (UVR). The stratospheric ozone layer, formed between 10 and 40 km from the surface, prevents almost all UVR of wavelengths less than 290 nm and a substantial proportion (70%-90%) of UVB radation from reaching the earth. Recent public and scientfic concern about ozone depletion and inecreased UV have lead to the establishment of many UV monitorng centers in the last few years. In 1980s less than 50 UV monitoring stations were operating around the word. Today more than 250 monitoring centers are underway for a variety of reasons (1). There are no systemic UV measurements reports in our China to date. In 1995, we start to measure ground-level solar radiation in shanghai area.

MATERIALS AND METHODS

UV-A and UV-B radiometer (Light and Electric Instruments Factory of Beijng Normal School). UV

radiation was measured in sunny days, from AM 9.00 to PM 3.00, total three days in a month, one is among the first ten days of a month, another is among the middle ten days of a month, the third is among the last ten days of a month. There are no any reflective object or shadow around mortoring point.

RESULTS

The highest means UVR irradiate value at noon is observed in July (UV-420, 6814 μW/cm^2; UV-365, 3184 μW/cm^2) or August (UV-297, 31.5 μW/cm^2) at shangai. The trend showed that radiation power of summer > that of autumn > that of spring > that of winter. The highest means UVR of a day appeared around noon. The UVA is nearly 100 times higher than UVB.

DISCUSSION

It is an important work to monitor the ground level UVR, especially for a long time. It can be to provide information to the public on UV levels and variations and to establish a basic UV climatology (2). It can also study cause and effects of UV transmission and detect long term variability.

The World Meteorological Organization (WMO) has established a global network called Global Atmosphere Watch (WMO).

It presently has eight observatory stations that make continuous spectral and broad band UV measurements.

The Global Environment Facility is supporting the creation of 10-15 additional stations in developing countries. Various national and multi-national agencies are also operating and establishig UV monitoring networks

In our operation, we realize that only litter information can be obtained from manual monitoring, because data was limited by no continuous monitoring. It is very necessary to set up automatic monitoring station in our country.

Part 2.
A COMPARISON OF IN VIVO AND IN VITRO TESTING OF SUNSCREENS

Solar UVR is a main risk factor of skin aging and cancer. How to protect oneself from UVR become popular as awareness of UVR's harm. In recent years, many kinds of sunscreens appeared in our country how to evaluate the effectiveness of sunscreen, sun protection factor (SPF) was usually used to evaluate the photoprotective efficacy. The SPF is defined as the ratio of the least amount of UVB energy (MED) required to produce a minimal erytema reaction through a sunscreen product film to the amount of energy required to produce the same erythema without any sunscreen application.

Very little, has been done to evaluate the effectiveness of the commercially available sanscreens in our country. In this study, 8 commercial sunscreens and a 8% Homosalate standard substrate were compared on *in vivo* human, *in vitro* mouse skin and transpore surgical tape.

MATERIALS AND METHODS

1000 W xenon lamp (Light and Electric Instruments Factory of Shanghai) with a cut-off fliter WB 280/2mm (Colour Glass Factory of Shanghai) is used as solar simulator light source. UV-B radiometer (Light and Eleetric Instruments Factory of Beijing Normal School). Transpore surgical tape (Optometric Inc. US). SD mice were supplied by department of experimental animal, Shanghai medical university. Sunscreen was provided by cosmectic company.

Testing *In vivo*

Human sun protection factor (SPF) testing on a panel of 20 adult volunteers (6 females and 14 males) were conducted according to the Federal Register OTC Sumcreens Monograph of US (3). SPF is the ratio of Minimal Erytema Dose (MED) with sunscreens

to the MED without sunscreen. The skin type of 20 volunteers were type IV as are most of Chinese.

Testing *In vitro*

SPF was measured according Stockdale's (4). Mouse skin was obtained from dorsal area of 5 day old SD mice. The epidermis was removed from dermal layer by immersing the mouse skin in 60 °C water for 30 seconds. The epidermal sheet of 2.5 cm in diameter were mounted on thin UVB transparent filter. Sunscreens were applied to mouse skin with 2 mg/cm^2. SPF was the ratio of the transmitting radation through the untreated monse epidermal sheet to that of the treated.

According to Furgson's (5) method. Transpore surgical tape was used to test the SPF of sunscreens. The procedure of SPF measurement on the tape was just as on mouse skin.

RESULTS

The SPFs, based on human testing, of the sunscreens A, B, C, D, E, F, G, H were 2.16, 5.14, 6.85, 7.38, 8.31, 9.66, 3.5, 12.5 respectively

There are no significant differences between the SPF detected with human *in vivo* and *in vitro* on transpore surgical tape. The coefficient of correlation is 0.9817 between human testing and transpore surgical tape. The SPF of 8% HMS sunscreen detecting with human and tape were compared with SPF suggested by FDA, there are no differences (U=1.37, 1,69; P>0.05).

There are no significant differences between the SPF detected with human *in vivo* and *in vitro* on mice skin except for G (t=2.33 P<0.05).

The coefficient of correlation is 0.9866 between human testing and mice skin. The SPF of 8% HMS sunscreen detecting with human and mice skin were compared with SPF (4.47±1.14) suggested by FDA, there are no differences (U=0.9556, 0.9556; P>0.05).

There are no significant differences between the SPF detected with tape and that with mice skin. The coefficient of correlation is 0.9860 between mice skin and transpore surgical tape.

DISCUSSION

Sunscreens had long history in western country, but only appeared in China in 1990s.

A lots of consumer did not know much about the sunscreens, so it is very important to find a simple and valid method to evaluate the effectiveness of sunscreens and give advises to consumers.

In the present study, the 8 commercial sunscreens were tested by three different methods. The SPFs of these sunscreens ranged from 2.16 to 12.5.

Snnscreens with SPF 6 to 8 are suitable for Chinese people, according to the recommendation of FDA (6).

In vivo human skin testing is the most essential and reliable method for determining SPF of sunscreens. But it takes a long time and makes photodamage to the tested skin of subjects.

It is very useful to detect the range of SPF of sunscreens before testing on human skin. *In vitro* mouse skin and transpore surgical tape were used to measure the SPF of 8 commercial sunscreens.

The results showed a good correlation between the *in vivo* and *in vitro* methods. It indicated that it was possible to predict the SPF of sunscreens with *in vitro* testing. The testing with transpore surgical tape is more easier than that of mouse skin.

Part 3.
EPIDEMIOLOGICAL STUDIES OF SUN PROTECTION IN UNDERGRADUATES

Sun exposure and sunburn, parcularly in childhood, are important risk factors for skin cancer (7-9). In order to detertmine whether the youngsters are aware of the link between skin cancer and excessive exposure to sunlight and whether they know how to protect themselves from exposure to sunlight. Investigation were made to 368 undergraduates.

MATERIALS AND METHODS

Using a questionnaires, information was obtained about general conditions, history of sunburn, and diseases associated with sunlight, the awareness of UVR harmful effects and how to protect oneself. Sunscreen Testing: 40 female students, their skin type was IV, no light allergic or drug allergic history, are chosen from newcomers, A, B, C, D 4 groups were divided random with 10 people in each group. Sunscreen for UVB was used by group A, placebo was used by group B, sunscreen against UVB + UVA was used by group C, sunscreen for UVA was applied by group D, About $2mg/cm^2$ of sunscreen was applied to the skin. A photo was taken before and after three weeks militarily exercise respectively. Using computerized imagine analysis techniques to measure the density of colour of 5 points in photo. The higher of density of color, the more pale of the skin.

RESULTS

There are 368 students involved in study, male and female are 50% each, averages of age is 18.6 years old, range from 16 to 21 years old, 5 (1.4%) had light allergic, 52 (14.1%) had sunburn, 2 (0.5%) had diseases associated with sunlight. 89.4% people are aware of UVR harmful effects. 70.4% consider that UVR can lead to skin aging, 88.2% agree with that UVR is risk factor of skin cancer.

But only 13.6% take care of themselves away from UVR. 77.7% know about sunscreens, but only 17,7% understand the meaning of SPF. In military exercises, 12% of people applied sunscreens every day, 11.1% used sunscreens between times, 76.9% did not use anything.

There were significant differences on density of color of photo taken before military exercise compared with that of after military exercise.

It indicated that the skin color become darker after military exercise. There were no differences among A, B, C and D groups either before military exercise or after.

DISCUSSIONS

The investigation show that though majority of people realized the harmful effects of UVR, they neglected to protect themselves from exposure to UVR. The result was similar to that of investigation in western country (10-11). It is urgent to take some propaganda against UVR. It seems that the using of sunscreen in China is not popular as in western country.

In human being testing of sunscreens, the results showed that the sunscreens did not supply any protection against UVR. The main causes of that may be sunscreen diluted by sweat, which is not rare during the military exercises. It indicated that waterproof sunscreens should be applied when doing some athletics. Another reason may be too many steps in processing the photo to distinguish the small differences. So it will be better to use computerized image analysis technique directly to detect the color of skin.

Part 4.
EFFECTS OF ULTRAVIOLET IRRADIATION ON HUMAN SKIN-DERIVED KERATINOCYTES AND FIBROBLAST IN VITRO

UVA radiation has the capacity to damage several celular targets, including membranes, proteins and DNA, and the mechanism of such damage is believed to involve reactive oxygen species, which may have a variety of harmful effects, including the peroxidation of unsaturated lipids (12-13). It has been demonstrated previously that relatively large amounts of ultraviolet UVA can produce photodamage and it is believed that UVB plays a major role in the induction of photodamage and photocarcinogenesis. Recent, a study showed that even suberythemal doses of repetitive UVA may lead to photoaging of the skin. We studied the effects of UVA irradiation on human skin derived keratinocytes and fibroblasts by detecting a various antigen expressed by these two types of cells.

MATERIALS AND METHODS

CELL CULTURE AND UVA IRRADIATION. Kreratinnocytes and fibroblasts were cultured in 10 well slide. Seeding densities was $4x10^4$ cells/ml, just as 1000 cells per well. The medium for kreratinnocytes was KGM, and for fibroblasts was RPMI, and for mixed was 66% KGM/34% RPMI. 25μl each cell suspension was put on to slides in square dish, put PBS at side to keep moist. Next day, check the density of cell, then add 10 ml medium, after three days, the cells were taken for irradiation. Before irradiation, the medium was aspirated and 5 ml HBSS were added to the dishes. Then put the dishes under the UVA lamp with the lid open and were irradiated to 10 mins, 30 mins, 60 mins, 90 mins and 120 mins respectively. Immediately after irradiation, the HBSS were aspirated and 10 medium were added and the cells incubated for another 24 hours. After 24 hours, the cells were checked for morphology and viability, then wash with PBS 10 mins for three times, and dry at room temperature, and stored in -70°C freezer.

IMMUNOHISCHEMISTRY. The cells reacted with primary antihodies P_{34} (1:50), GB3 (1:200), Plakoglobin (1:100), 11-5F (1:5), 3EI (1:1000), G71 (1:100), Coll I (1:50), Coll III (1:50), Coll IV (1:50), Coll VII (1:10), Fibronection (1:100), FSP (1:200) for 60 minutes at 37°C. After 10 minutes wash for three times, the cells reacted with FITC labled secondary antibodies for 30 minutes at 37°C. After 10 minutes wash for three times, the slide was mounted with citiflour. The slide was observed on olympus microscope by three individual. The density of fluorescence in cells was recorded as –, 1+, 2+, 3+, 4+.

RESULTS

The results showed that as the dose of UVA irradiation increase, the degree of staining in all cells become decrescent.

Especially for Coll I and Coll III, when the dose of UVA were 3 J/cm^2, the staining faded, and the dose of UVA were 9 J/cm2, the staining disappear.

DISCUSSION

Dermis contains predominantly type I collagen, with lesser amounts of type III collagen. The individual polypeptide chains of types I and III collagens are synthesized by dermal fibroblasts, as precursor molecules, procollagens, which contain globular amino and carboxy terminal domains. Within the cell, the individual chains assemble into trimeric type I or III procollagens, which are secreted into the extracellular space as soluble proteins. During formation of insoluble type I collagen fibrils, the carboxy and amino terminal domains are cleaved by specific protease, giving rise to pN collagen and pC collagen, respectively, which assemble into thin fibrils (14). Recent study showed that type I and type III collagen precursor levels are signficantly reduced in severely photodamaged human skin (15-16). Our results demonstrate from cellular level that UVA was a risk factor of skin aging.

Part 5. THE EFFECT OF UVR AND UROCANIC ACID ISOMERS ON DELAYED TYPE HYPERSENSITIVITY IN MICE

Irradation with ultraviolet B suppresses some cell-mediated immune responses to a variety of antigens, including contact sensitizers (17-18). Following UV irradiation there is modulation of Langerhans cells markers and keratinocytes are induced to synthesize and secrete tumor necrosis factor-α (TNF-α) (19). Cis-urocanic acid (cis-UCA) has been suggested as a photoreceptor for UV and has been demonstrated to suppress immune responses in several experiment (20). In the present study the effects of UVR irradiation on delayed type hypersensitivity (DTH) in mice were compared with that of cis-UCA.

MATERIALS AND METHODS

Urocanic acid, Ovalbumin and DNFB (Sigma). UV Lamp (Light and Electric Instument Factory of Shanghai). Antigens of DNP_6OVA were prepared according to Yano's (21) methods. SD mice were divided into A, B, C, D, E and F 6 groups with 10 in each group. A is control. B injected with trans-UCA (200 mg), C injected with cis-UCA (200 mg), D injected with cis-UCA (400 mg), E injected with cis-UCA (600 mg), F irradiated with UVR as a single dose of 5 kJ/m^2.

The mice were tested for DTH to DNFB 7 days after sensitization. Ear thinckness were measured before the mice were challenged by injecting 10 μl of antigen into each ear pinna. The ear thicknesses were again measure per mouse. Suppression of DTH was determined by the formula: % suppression = (1-net increase of experimental mice/net increase of control mice) x 100.

RESULTS

The suppression of DTH in group of C, D and E were 47.3%, 52.5%, 56.0% respectively. There are dose-response relationship ($r=0.9820$, $t_r=5.196$, $P<0.05$). There are no significant differences between control A and B. There are significant differences between C, D, E, F and A ($t=23.13, 20.25, 44.67, 19.47$, $P<0.001$). There are significant differences between C, D, E, F and B ($t=14.70, 15.99, 18.37, 7.70$, $P<0.01$). There are significant differences between D, E and C ($t=2.309$, $P<0.05$; $t=4.768$, $P<0.01$). There are significant differences between D, E and F ($t=3.36, 5.399$, $P<0.01$).

DISCUSSION

There are two subpopulations of T helper cells, designate Th1 and Th2 which appear to be differentially affected by UV exposure. These two populations are thought to regulate different sets of immune responses. Th1 cell produce IL-2 and γINF as well as other cytokines, promote DTH responses such CHS, provide help for certain antibody subtype responses including complement-fixing antibodies, activate macrophages, and may be particularly important for dealing with antigens expressed on cell surfaces, such as viral and tumor antigens (22). Th2 cell produce a different array of cytokines including IL-4 and IL-5 which promotes antibody responses. UVR causes the release of mediators from the skin which alter the antigen presenting capability of Langerhans cells as well as antigen presenting cells at other sites, resulting in the development of suppresser T-cell. It may be that these suppresser T cells are Th2 cells. The net result is the failure to activate Th1 cells and suppression of DTH responses thought to play an important role in host defences against certain types of tumors and microbial infections. The immune suppression is antigen specific and is long lasting. In previous studes, using a single dose of urocanic acid, in present study, three doses were applied. The result show good relationship of dose-responses. The effects of UVR irradiation was similar to group C (cis-UCA 200 μg).

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SUNSCREEN FORMULATION: A STUDY OF SILICONE-EMULSIFIANT CONCENTRATION

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Synopsis

Silicone surfactants can be used to prepare emulsions at room temperature, and yiels stable W/O and O/W emulsions with excellent appearance and optimum hydrating and protective properties. Silicones impart substantiveness to the preparation. This was confirmed experimentally in washing-off tests. After 24 h of testing, an acceptable percentage of the initial concentration of sunscreen remained present in the formulation.
Physical stability assays (centrifugation and aging at 25°C, 40°C and 60°C) yielded similar results. Rheological assays, tests at different pH values, and droplet size studies were also done. Stability of the preparations was adequate, pH showed minimal variations, and droplets remained small and homogeneous.

Riassunto

I tensioattivi al silicone possono essere utilizzati per preparare emulsioni a temperatura ambiente ed emulsioni A/O e O/A dall'aspetto eccellente ed ottime proprietà idratanti e protettive. I siliconi conferiscono consistenza alla preparazione. Questo è stato confermato sperimentalmente nei test di washing-off. Dopo 24 ore di test una percentuale accettabile della concentrazione iniziale dello schermo solare rimaneva presente nella formulazione.
Le analisi sulla stabilità fisica (centrifuga e invecchiamento a 25°C, 40°C e 60°C) hanno dato simili risultati. Sono stati altresì eseguite analisi reologiche, test a diversi valori di pH, e studi sulle dimensioni delle gocce.
La stabilità dei preparati era adeguata, il pH ha indicato variazioni minime e le gocce sono rimaste piccole e omogenee.

INTRODUCTION

Advances in silicone chemistry have led in recent years to the development of a wide variety of compounds derived from the basic structure of siloxane bond polymers (1-3).

One type of new compounds are the silicone surfactants with groups of ethylene oxide (EO), propylene oxide (PO) or copolymers of both (EO/PO). W/O and O/W surfactants are defined according to the proportions of EO or PO they contain (4). The advantages of silicone emulsions are that they can be prepared at room temperature, they have adequate extensibility, and do not leave a greasy, sticky, or unctuous film on the skin. These emulsions contain approximately 80% water and are thus of the greatest interest economically (5). The present study on sunscreen formulation was designed with different concentrations of W/O (DC 3225-C) silicone surfactant. The emulsion is prepared at room temperature, the formulae contain almost no greasy or oily components, which imparts the best dry emollient characteristics, and hydratant properties with a photoprotector type screen (titanium dioxide) to be used by people needing total protection from solar radiation, particularly children, people with alipic skin, etc. (6-7).

Physical stability assays of the preparation were made at 25°, 40° and 60°C, and resistance to centrifugation was also used to test stability (8,9).

MATERIALS AND METHODS

- Tioveil AQN (titanium dioxide in aqueous dispersion), supplied by Comercial Quimica Massó (Barcelona) is an oily sunscreen.
- DC 3225-C (a mixture of cyclometione and copolyol dimethicone), a W/O surfactant, and DC-344 (tetrameric cyclomethicone) were supplied by Dow Corning.
- Isopropyl myristate, glycerin and sodium chloride was obtained from Panreac.
- Distilled water was used in all formulations.

Composition of Formulations

The formulation tested is:

	A	B	C
Tioveil AQN	5	5	5
DC3225-C	3	5	8
DC-344	5	5	5
Isopropyl myristate	12	12	12
Glycerin	3	3	3
Sodium Chloride	1	1	1
Distilled water		c.s. 100	

PREPARATION OF THE EMULSION

The galenical formula was developed in accordance with recent technology for preparing emulsions at room temperature.

1. Mix oily components and aqueous components separately.
2. Add the aqueous phase to the oily phase very slowly with rapid shaking at 1700 rpm (ultra-Turrax, T25 IKA labortechnik) under a vacuum if possible, until a milky, homogeneous emulsion forms.
3. Homogeneize in a colloid mill (Lancor-Himmel, 2G.80 1.1.H Bilbao, Spain).

RESULTS AND DISCUSSION

Spectrophotometric Analysis

Fig. 1 illustrates the UV absorption spectra of Tioveil AQN. There is no pronounced peak absorption, indicating the tioveil to be a sunscreen with a protective effect across the entire ultraviolet range, with a slight increase at 375 nm.

Similar effects are obtained with our formulations

(Figs. 1 A,B, and C) which showed the most absorbance for formulation C (the higehest surfactant concentration).

Wash-off Tests

Since the formulations were designed as sunscreens, it was necessary to ensure that the emulsion provided adequate substantiveness.

For 24h, samples were subjected to a water flow at 25°C. The percentage of sunscreen released from the sample is shown in Fig. 2 for different periods. The values are obtained at 375 nm wavelength, where the tioveil showed a slight peak.

After 24 h (i.e. much longer than under conditions of normal use), a maximum of approximately 2-8 % of tioveil had been lost from the formulation. This result indicates that a significant proportion of the filter remains on the skin even after repeated bathing, making it without doubt a highly water-resistant formulation.

The formulation with the least surfactant (A) (the most fluid) showed the most liberation of tioveil, probably due to the low viscosity of the formulation.

Stability Analysis

Since the preparations were thermodynamically unstable, we investigated viscosity and droplet size as well as the organoleptic characteristics of the formulations.

Tests were run at different temperatures in accordance with bibliographic references. Samples were kept at room temperature (25°C) for over six months, at 40°C for 3 months, and at 60°C for two weeks. We then measured pH to check for variations over time, finding the pH values to remain near 7±0.5 for most of the experimental period. Variations in temperature and over time were minimal, indicating that the preparations are stable.

A series of rheological tests was done with samples at room temperature, at 40° C and at 60° C. Fig. 3 shows rheograms for the beginning of the study and Fig. 4-6 shows rheograms for samples followed for six months at room temperature, 3 months at 40°C and two weeks at 60°C, for formulation A,

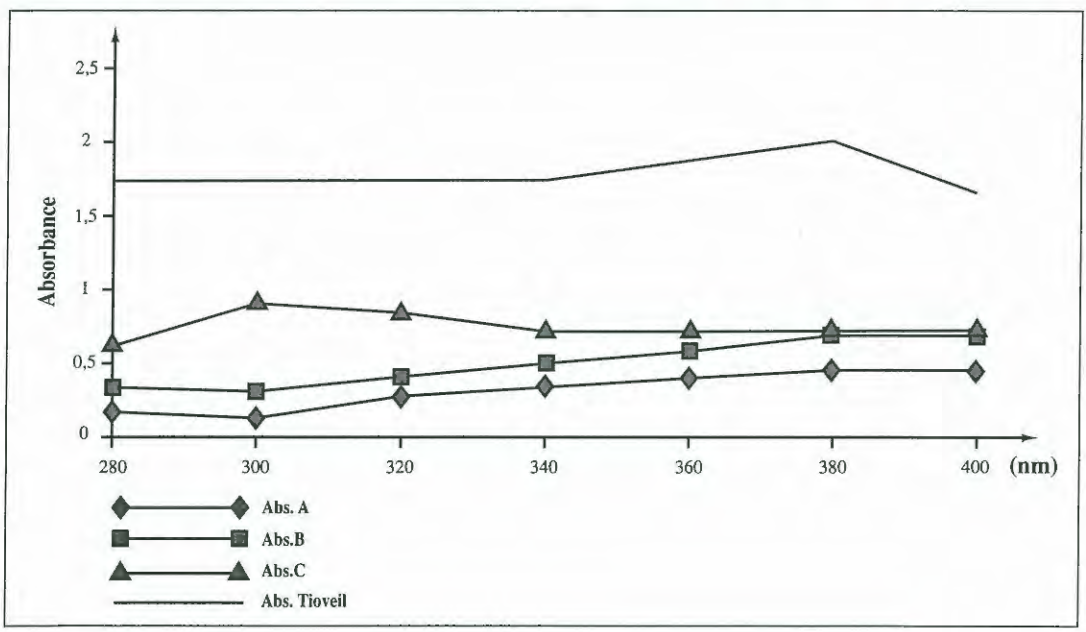

Fig. 1. Absorption spectra of Tioveil AQN and formulations A, B and C.

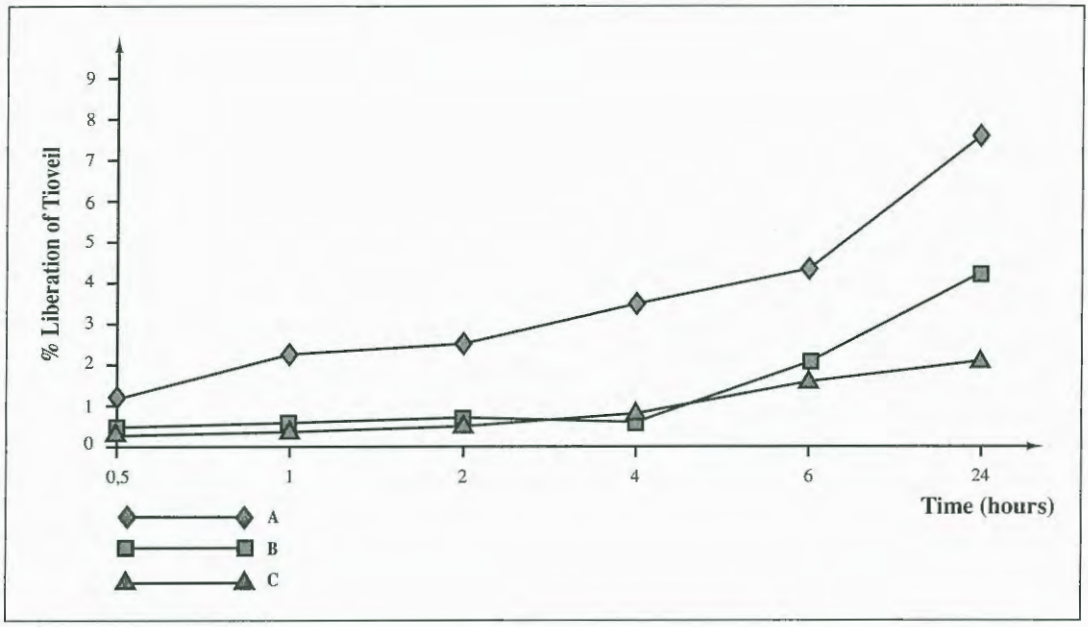

Fig. 2. Percentage of Tioveil AQN released by the formulations A, B and C as a function of time.

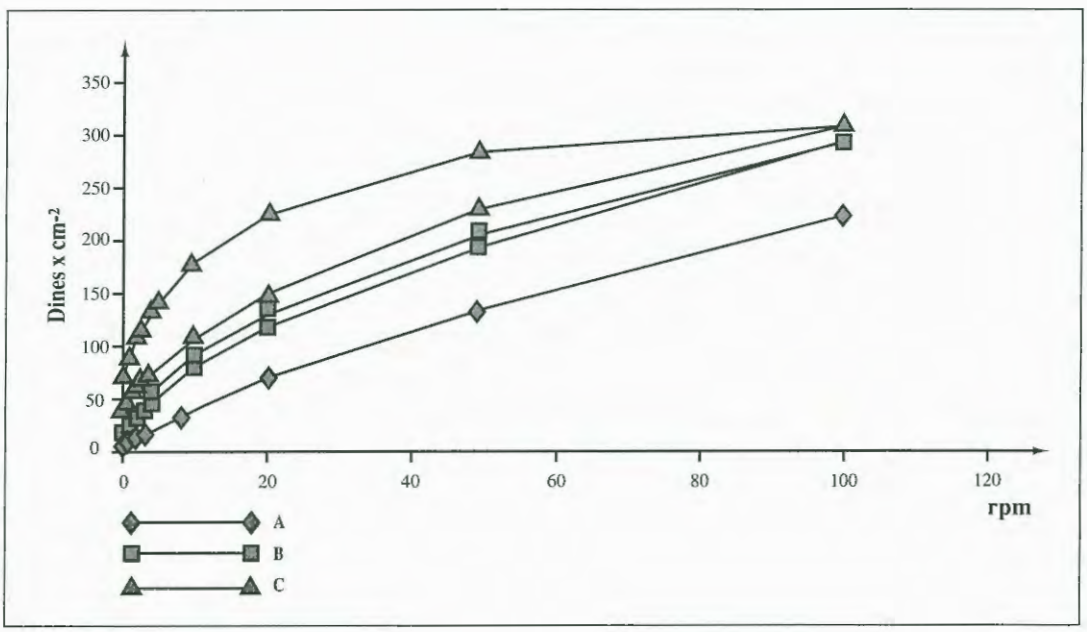

Fig. 3. Rheograms of the formulations A, B and C.

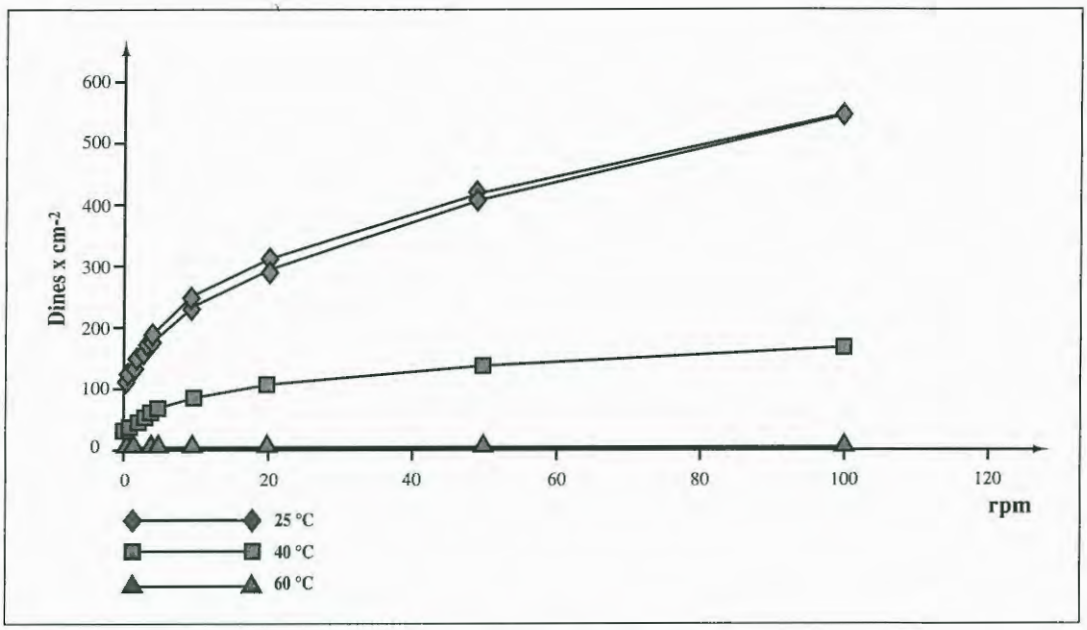

Fig. 4. Rheograms of the formulation A at room temperature 400C and 600C.

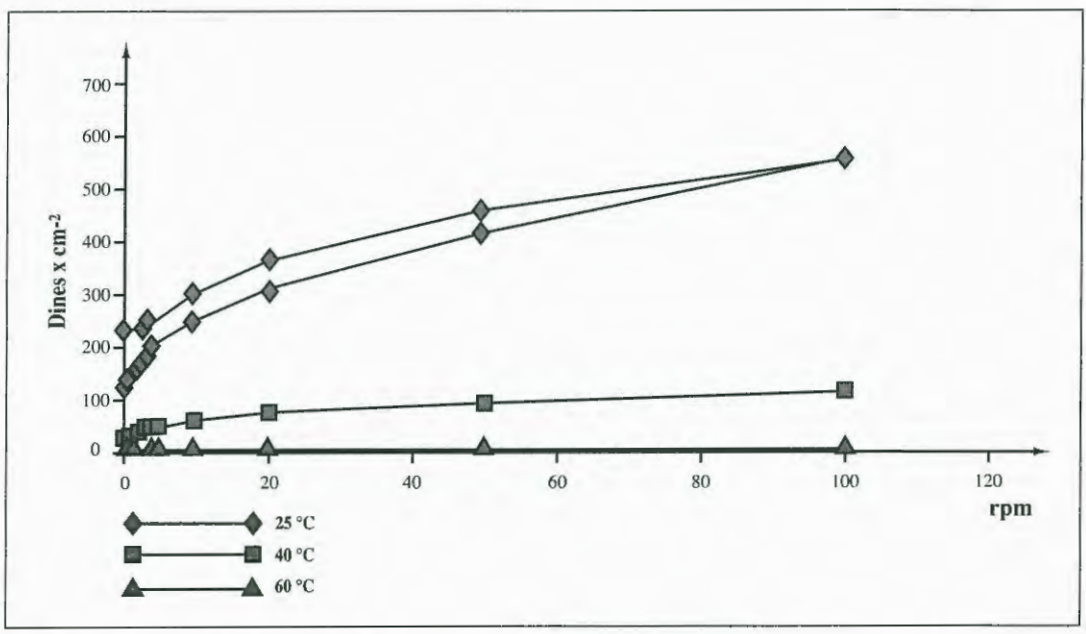

Fig. 5. Same as Figure 4 at formulation B.

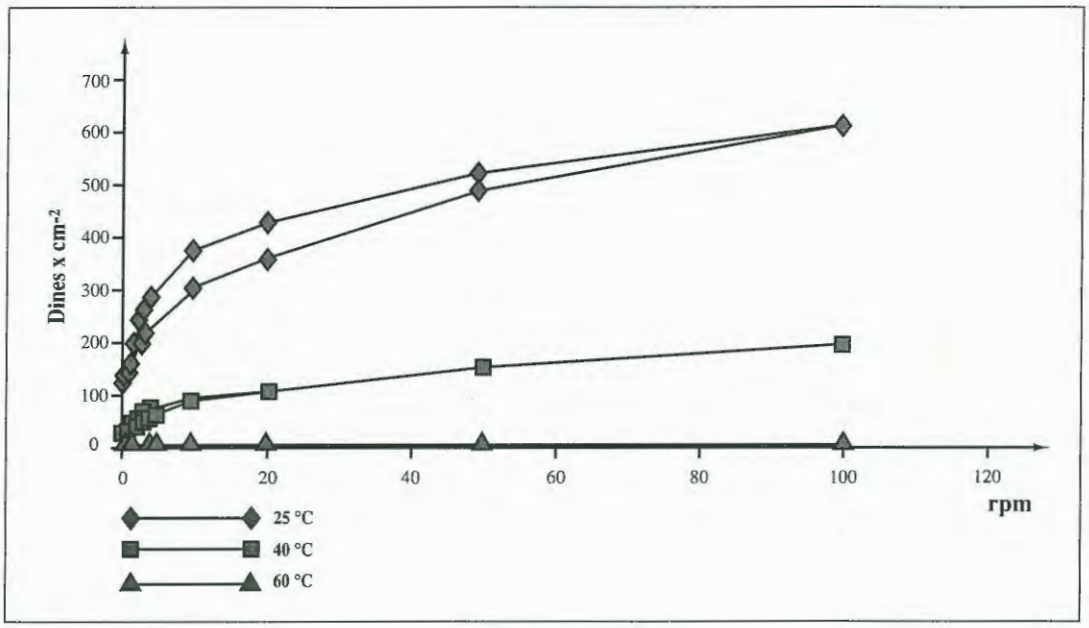

Fig. 6. Same as Figure 4 at formulation C.

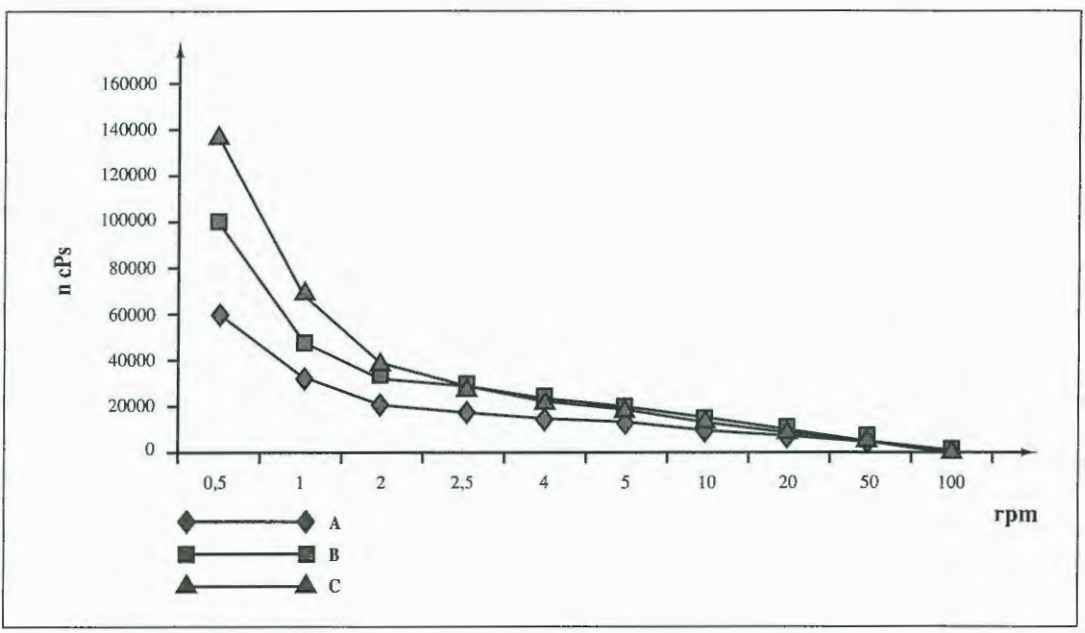

Fig. 7. Changes in viscosity with shear rate in formulations A, B and C.

B and C respectively.
The room temperature rheograms for the three formulations were similar, showing the plastic behaviour characteristic of these preparations. Note the higest deformation force in formulation C. A hysteresis cycle was most prominent in samples with the most surfactant.
The viscosity values showed an increase in surfactant concentration, notable at low shear rate, with nearly identical values from 10-100 rpm (Fig. 7).
These results were verified experimentally with microscopic examination that confirmed the stability of the samples, particularly of sample C (the most concentrated surfactant).
Droplet size remained small and homogeneous, although during the final days of experiment samples at 60°C showed a slight reduction in particle size, with a slight loss of homogeneity in comparison with the beginning of the experiment.
Scarce coalescence was also observed in this samples. Figures 8 and 9 show droplets from sample A that were kept at room temperature (photo 8) for 6 months and at 60°C (photo 9) for two weeks. Resistance to centrifugation was also used to test stability.
This procedure is useful for comparing formulas of similar composition and density on the basis of phase separation, creaming, or exudation. In accordance with bibliographic references, samples were centrifuged at 3000 rpm for 30 min.
None of the tests caused separation of the components in the three formulations, confirming once again the stability of the preparations.

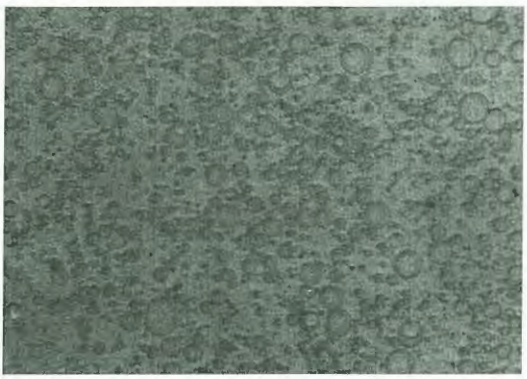

Fig. 8. Optical micrograph of the formulation A at room temperature.

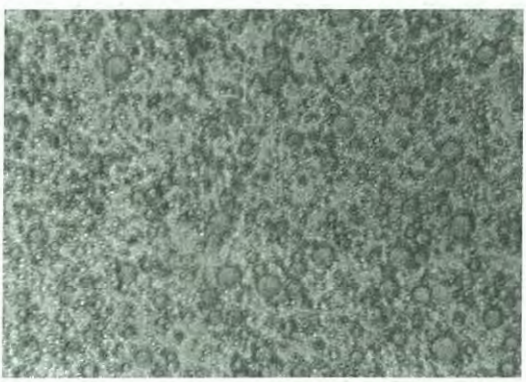

Fig. 9. Same as Figure 8 at 60°C.

CONCLUSION

Of the three formulations studied, all were stable during the time of experiment.
Nevertheless, it is apparent that formulation C has the best organoleptic characteristics, the best substantiveness and the best viscosity, with adequate extensibility.

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A NEW DIFFUSION SYSTEM THROUGH THE MUCOUS MEMBRANES, SKIN AND HAIR

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Key words: *Mucous membranes, Skin, Hair diffusion system, MDS, Skin hydration, Keratin, Absorption, Penetration, Skin barrier, Lipids.*

Synopsis

Although mucosae, skin and hair are complex systems that have a common origin, they are different thanks to the presence of mucous, of surface lipidic film and a greater or lesser quantity of keratin.
In order to obtain an optimal cosmetic penetration through this kind of tissues, it is fundamental the substantial modification of the vehycles employed.
To achieve this goal a particular DIFFUSION SYSTEM (MDS®) was developed. This MDS® can be considered the starting point of differentiated vehycles.

Riassunto

Sia le mucose che la cute o i capelli rappresentano sistemi complessi, che pur essendo di origine comune sono dissimili tra di loro per la presenza rispettivamente del muco, del film lipidico di superficie e di una maggiore quantità di cheratina.
Naturalmente per ottenere una penetrazione attraverso tali tessuti, che sia ottimale con l'uso cosmetico, è necessario modificare sostanzialmente i veicoli utilizzati. È stato perciò messo a punto un particolare DIFFUSION SYSTEM (MDS®) in grado di rappresentare la base di partenza di veicoli differenziati.

Mucous membranes are highly specialized ephitelial tissues, totally different from the skin that shares the interest of gynaecologists, dermatologists, cytologists and genito-urinary surgeons.

There are three main differences between mucous membranes and body skin.

The first is the fact that the former is generally covered by a protective and lubrificant mucus over its surface whilst the skin produces continuous and impermeable lipid-filled keratin layers which vary from site to site, and are not modifiable by the presence of the surface lipids film (1).

The second major difference is the sensivity of mucous membranes to circulating hormones, whereas human skin is virtually unaffected by androgenic or aestrogenic hormones until old age is reached.

The third difference of mucous membranes is their turnover rate which is much greater than that of the skin epidermis.

Because of the continous presence and activity of mucus even the keratinized zones of mucous epithelia are maximally hydrated and therefore would be expected to show an incresead permeability to water compared with body skin.

Thus it was recognized that the lipid-filled, intercellular domains is crucially important to barrier function of the skin, such as mucus performs the barrier function of mucous membranes.

For these reasons, cosmetic raw materials and active ingredients used can move through epithelia by simple diffusion, endocytosis or by active transport across membranes to obtain a real cosmetic diffusion system.

It is of most importance the chemical and physical nature of the penetrating selected active ingredient in relation to its movement through the vaginal mucous membranes.

In general, ions have more difficulty in penetrating than molecules, but small molecules, such as glycine or vitamin A, penetrate more easily than larger molecules (2).

Thus, the degree of ionization of an active substance will affect the rate at which is often dependent upon the pH. Moreover generally speaking, the permeability of mucous membrans is similar to that of fully hydrated body skin.

After the evaluation of all these parameters new vehicles have been developed, called Diffusion System® (MDS®), for mucous membranes, skin or hair.

What is the difference between skin and mucous membranes? The skin is covered by a dead durable, highly linked and lipid-filled protein keratin which forms a protective layer against the external environment, the Stratum Corneum (SC).

This "bricks and mortar" structure where the major lipids are between the cells forming the mortar, acts as a barrier against the penetration of organisms and other unwanted materials (3).

Moreover topically applied physiologic lipids cross the SC and enter the nucleated cell layers, followed by an incorporation into lamellar body secretory system.

Because metabolic processing is required, the impact of these lipids on barrier recovering is delayed of about two hours. The ability of these lipids to either worsen, normalize or accelerate barrier recovery rates in human epidermis is dependent on the ratios of the key lipids applied, ceramides, free fatty acids and cholesterol and, of course, on the age of the treated people (4).

Variation in the composition and proportion of these three lipid families can lead to either deterioration, normalization or acceleration of barrier repair.

A ceramide-dominant system, for example, would accelerate barrier recovery in chronologically aged skin, as it does in young skin, whether meanwhile cholesterol alone delays in chronologically aged skin, consistent with the marked abnormality in cholesterol synthesis in aged epidermis (5,6).

On the other hand, the unkeratinized mucus epithelia have living cells on their surface which would be very vulnerable to attack by micro-organisms and to the effects of toxic molecules where it not for the presence of mucus (7).

Moreover, the hair is modified epidermal cells composed of keratin, the main component of the horny layer in the skin. In biochemical terms keratin is a proteinaceous material where numerous s-s bonds create a framework between peptide chains resulting in very low solubility.

In histological terms, it is composed in three parts: keratin fibres, interfibrous materials and horny intermembrane materials.
However, in the keratinization process of forming hair, the cells are not all alike; the medulla, the cortex, the cuticle and the inner root sheath differentiate into cells with characteristic morphology and they each have characteristic forms of keratin.
The cells forming the outer root sheath are very similar in the form to the basal cell layer and spinous cells of the epidermis.
Cuticle has a rough surface composed of hard keratin protein; it is easily worn off by excessive brushing or strong shampoo and it is subject to the greatest environmental stresses including dry atmospheres, UV-light, sea water, swimming-pool chlorine, etc.
In particular, the cuticle of the hair shaft is directly affected by these stresses resulting in several cumulative types of damage.
For this reason hair splits and breaks easily. The penetration through all the tissues, the skin and the mucous membranes or the hair, is enhanced by the phenomenon of hydration.
What is interesting to remember is the protective action of mucus depending on its physio-chemical characteristics and on the specific, highly active macromolecular system such as immunoglobulins and other substances which it contains (8,9).
In fact mucus is a solution of a number of polymers which behaves quite differently from a solution of small molecules, when two unlike polymers in solution are mixed they tend to remain as two distint solutions, whereas solutions of two macromolecular systems intermingle.
Thus, in the first case, it may be said that the one polymer is insoluble in the other, and in the second case, each is *soluble* in the other's solution.
This phenomenon seems to be well connected to the normal forces existing between molecules, such as hydrogen bonding, Van der Waal's forces, and hydrophobic interactions, which are usually stronger between like molecules than between unlike molecules.
A special Diffusion System® (MDS®) (Fig. 1) was studied to improve and to facilitate the absorption

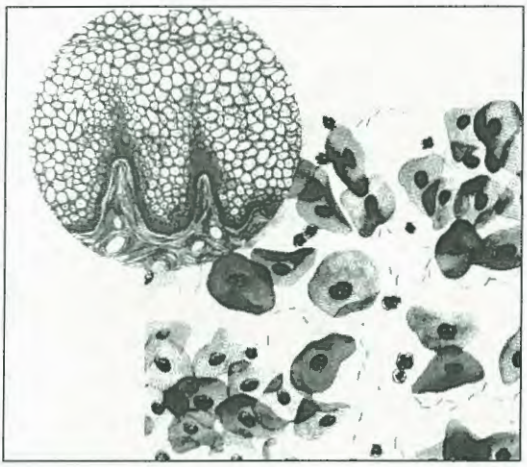

Fig. 1.

of active raw materials through the skin and the mucous membranes for the vehicle formulation of gynecological and/or dermatological products.
Have been selected specific macromolecules which have a structure compatible with the molecules of the mucus glycoprotein, thus enabling them to enter the environment of the mucus layer (10).
Moreover the vehicle used allows also smaller active molecules to interchange between the living epithelial cells and their surroundings, depending on their molecular size and ionic charge (11, 12).
This is the reason of the patented use of gelatin-glycine and/or gelatin-arginine, or gelatin-cystine necessary for enhancing both the hydration of mucus membranes and penetration rate of the active compounds (13-15).
Because of this all the components of MDS® are miscible with mucus and migrate to the outer surface of the mucus where they form a monolayer with the mucus-like portion of their molecules in the mucus phase and the other portion protruding into the surrounding medium.
These may be the reasons of the demonstrated clinical activity of some gynaecological cosmetics such as Elageno® A Monodose or Elageno® A Gel (15-18). They form an organized defensive barrier at the interface between the epithelia surface and its environment and mantain the normal pH values,

Fig. 2. Before treatment with a cationic-conditioner

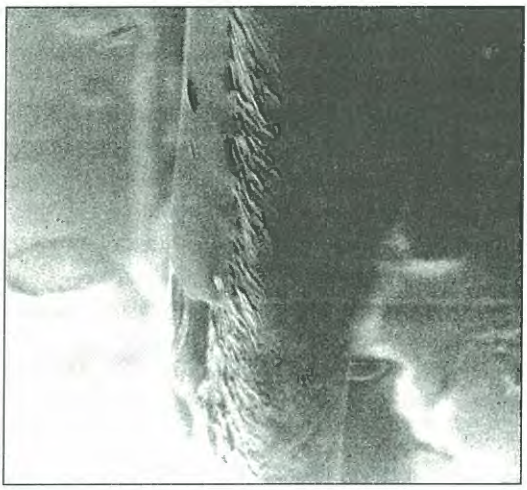

Fig. 3. After treatment with a cationic-conditioner

protecting the underlying cells against bacterial and viral infections. Contemporary throughout the activity of the vitamin A and PCA, Elageno® A seems to stimulate or induce also the formation of mucoproteins and other mucus substances.

Moreover, to reduce the damages of the hair it is useful to use, for example, cationic surfactant molecules to be absorbed on the hair surface. The hydrophilic group of the cationic surfactant faces towards the hair and is absorbed on to it electrostatically, while the lipophilic group is oriented outward. As a conseguence, the hair surface is covered by lipophylic groups making it smooth and protected (Fig. 2 ,3).

This is one of the principles the MDS® is based on. The system has been developed also for the formulation of hair products, such as shampoos, hair conditioners and lotions (19-20).

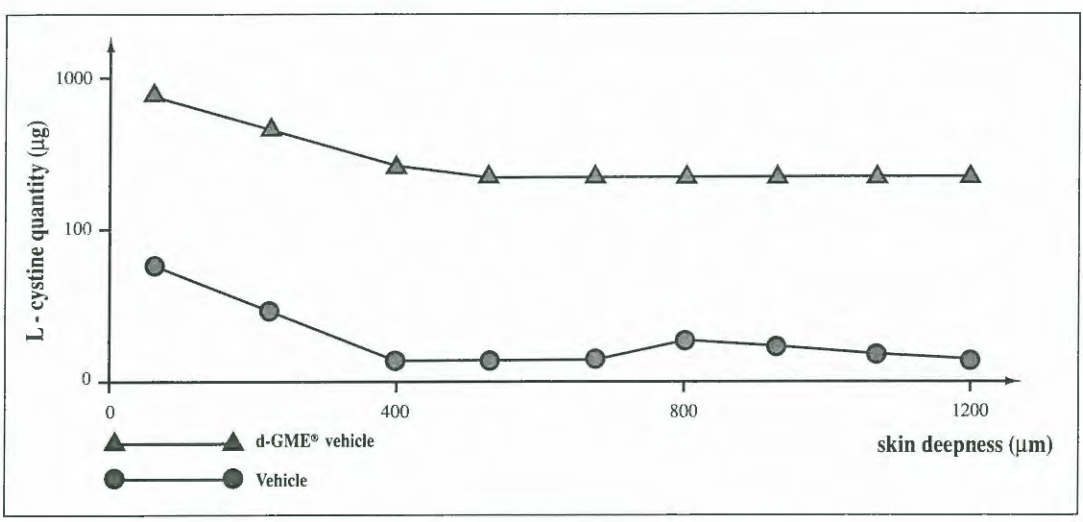

Fig. 4. Average L-cystine distribution in the scalp in function of cutaneous deepness. After one topical application (20 stripping)

Through the use of special oils, quaternized compounds and molecules that enhance the penetration (Fig. 4) became possible the formulation of new shampoos suitable to remove dirt, without damaging the protective lipidic film that covers both the scalp and the hair and capable to protect the hair from environmental damages while improving hair turnover by extending their anagenic cycle.
Thanks to a simple phenomenon of molecular chemistry active ingredients are made through the MDS® perfectly similar to hair keratins.
Thus they immediately fix to hair locks and leave on the hair an even film of substances that keep nourishing and protecting it until next washing.
What is of most interest is that this new DIFFUSION SYSTEM developed by our Research Team proved to be extremely adaptable and consistent with any cosmetic formulation requirements nowadays.

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PROSPECTS FOR CUTANEOUS WOUND HEALING IN AGEING SKIN.

A WORKING HYPOTESIS: CHITOSAN AND CERAMIDES

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Key words: *Ceramides, chitosan, wound healing*

Synopsis

In elderly people the cutis is characterised by structural fragility due to causes ranging from the thinning of the lipid-epithelial stratum to reduced dermal vascularisation, in particular, reduction in the content of all lipids - due mainly to sphingolipids and ceramides, especially at the level of the stratum corneum - and in the extracellular matrix because of greater degradation and reduced synthesis.
We set out to investigate the functional implications of a molecular association of lipopolysaccarides applied to elderly people wound healing with a veiw to re-estabilishing the cutaneous microenvironment which in these subjects is unbalanced and at risk. Water-soluble chitosans in the class of N-carboxyalkyl chitosans possess antimicrobial activity including candidacidal activity, and regenerated chitins and water-soluble chitosans are being used as wound dressings which facilitate the formation of new, ordered tissue. Based on these observations, the combined action of ceramides and chitosan appears to meet the requirements of the elderly, and may constitute *in vivo* a natural association capable of improving the cutaneous microenvironment.

Riassunto

Negli anziani la cute si caratterizza per una fragilità strutturale principalmente dovuta ad un assottigliamento dell'epitelio, del film lipidico di superficie e ad una riduzione della vascolarizzazione.
In particolare la riduzione del contenuto di lipidi a livello dello strato corneo e della matrice extracellulare si deve ad una maggiore degradazione e ad una ridotta sintesi principalmente di sfingolipidi e ceramidi.
È nostra intenzione condurre uno studio per valutare le implicazioni funzionali che una nuova associazione di lipopolisaccaridi potrebbe avere nella cicatrizzazione di soggetti anziani al fine di ristabilire il microambiente cutaneo che in essi è alterato e a rischio. I Chitosani solubili della classe degli N-carbossialchilchitosani, che possiedono attività antimicrobiche e anti-candidosiche, sono già stati utilizzati come presidi per la cicatrizzazione favorendo una ordinata riparazione dei tessuti.
L'utilizzo di un presidio costituito da ceramidi e chitosani sembra rispondere alle esigenze della cute dell'anziano costituendo un'associazione probabilmente in grado di migliorare il microambiente cutaneo.

As major costituents of intercellular lipids, sphingolipids are important determinants of stratum-corneum water holding and permeability barrier function (1). The epidermal permeability barrier is therefore provided by intracellular lipids forming multiple membrane bilayers in the stratum comeum. An alteration in the lipid balance should thus be considered a predisposing factor for dry skin, which in the elderly is often the cause of itching sine materia (2).

In elderly people the cutis is characterised by structural fragility due to causes ranging from the thinning of the lipid-epithelial stratum to reduced dermal vascularisation, in particular, reduction in the content of all lipids - though mainly of sphingolipids and ceramides, especially at the level of the stratum corneum - and in the extracellular matrix because of greater degradation and reduced synthesis (2).

In this medico-cosmetological context, we set out to investigate the functional implications of a molecular association of lipo-polysaccharides applied to the cutis of elderly people with a view to re-establishing the cutaneous microenvironment which in these subjects is unbalanced and at risk. Previous experience on tissue repair features in elderly subjects indicate that their "thin skin", especially in women with (post-menopausal) estrogen deficit, while showing slow epithelial and fibroblastic proliferation, usually allows the stromal matrix to be repaired without antiesthetic scars.

In these subjects precarious skin hydration constitutes both cause and effect of tissue dystrophisms which depend on its reduced reactivity to damage and on the gradual thinning of the hydrolipidic stratum (2).

Based on these observations, the combined action of ceramides and chitosan could appears to meet the requirements of the elderly, and might constitute *in vivo* a natural association capable of improving the cutaneous microenvironment of these and other subjects (!).

Interestingly, sphingolipid metabolities participate in key events of signal transduction and cell regulation. In the sphingomyelin cycle, a number of extracellular agents and insults (such as tumour necrosis factor, fas-ligands, and chemotherapeutic agents) cause the activation of sphingomyelinases, which act on membrane sphingomyelin and release ceramide (3, 4).

In the first place, membrane glyco and sphingolipids are important in the modulation of activity, adhesion to the substrate and cell morphology; indeed, the morphological differentiation of the epidermis rests on a high ceramide content (5). We thus set out to test a cosmetological combination of ceramides, which act prevalently at epidermal level, with chitosans. As regards the latter, similarities between some modified chitins and hyaluronic acid have been reported and the morphogenetic role of glycosaminoglycans is known to lead to cellular replication, stromal collagen network and biofunctional characteristics close to normal.

Chitosans are polysaccharides derived from chitin, which as raw material is amply available and comes from a number of crustaceans. It can be described as a copolymer of N-acetylglucosamine and D-glucosamine. Medical-grade chitins and chitosans are commercially available. To enhance their solubility, they can be chemically or enzymatically modified based on mild treatments in aqueous media, leading to highly purified products. This is particularly true of the modified chitosans in the class of N-carboxyalkyl chitosans (7).

Further properties of these molecules are: a) high capacity to adsorb water, the modified chitosans being able to modify the water structure, b) absence of noxious effects on healthy tissues, and c) reparative action on wounded tissues.

Water-soluble chitosans in the class of N-carboxyalkyl chitosans possess antimicrobial activity including candidacidal activity, and regenerated chitins and water-soluble chitosans are being used as wound dressing which facilitate the formation of new, ordered tissue. Furthermore chitosans are chelating agents and prevent the adsorption of toxic heavy metal ions accidentally present in solutions (7).

In laboratory animals, N-carboxybutyl chitosan, a water-soluble chitin derivative, has been demonstrated to induce the formation of ordered repair tissue where collagen bundles had a regular direction; wounds treated with N-carboxybutyl chitosan did not show evident

scar formation or wound contraction.
The results of some of our studies - confirming the data discussed above - are reported below (6).

PATIENTS, CLINICAL ASPECTS AND MORPHOLOGICAL INVESTIGATIONS

Four plastic-surgery patients, two males and two females, aged 30 to 50, underwent medium-thickness dermo-epidermal square explants (50 cm^2) performed with the aid of a dermotome on the frontal part of the thigh. This donor site was treated with N-carboxybutyl chitosan (area A). A similar explant was made close to it (control site, area B) and treated with phytostimuline gauze. Both sites were medicated after 7 days. On days 10 and 30, when the surgical wounds were clinically healed, biopsies were obtained from both areas.

N-carboxybutyl chitosan

Medical-grade chitosan was obtained from Alaska king crab chitin and was further modified into water-soluble N-carboxybutyl chitosan according to our own procedure (6). The resulting solution was dialysed and freeze-dried to produce soft and flexible pads which were sterilised and applied to the wound. Analytical data were: M_w 720,000, determined by laser light-scattering spectrophotometry; degree of N-carboxybutylation 0.27, determined by high-pressure liquid chromatography; ashes at 600°C, <0.1%, by gravimetry, and pH of the 1% solution 6.2.

Transmission electron microscopy investigations

Specimens were fixed in 2.0% glutaraldehyde in 0.15 cacodylate buffer, post-fixed in 1% OSO_4 in cacodylate buffer and dehydrated in increasing concentrations of ethanol. The samples were embedded in Araldite. Semithin and ultrathin sections were cut using a Reichert Ultracut E microtome; ultrathin sections were counterstained with uranyl acetate and lead citrate and observed with a Zeiss EM 109 electron microscope.

Clinical aspects

One of the advantages of using N-carboxybutyl chitosan in wound management is that it gelifies in contact with the wound fluids, forming a layer which provides an outstanding protection of the newly-formed tissues against mechanical damage. The outer surface of the pad acquires the aspect of a crust and provides protection against secondary infections by virtue of the bactericidal action of the polymer. Infections were not observed in any of these fours patients.
During the healing period, the square shape of the wound was preserved, though its size decreased progressively, while in control wounds the square shape was soon lost after traditional medication. Complete healing occurred after 8 days for area A and 7 days for the control area in all patients.

Morphological investigations

- **Repair process in control areas.**
  Ultrastructural analysis evidenced the presence of repair tissue showing a clear mesenchymal cell component represented by polygonal elements (i.e. fibroblasts) and a small number of inflammatory cells. The cell distribution was irregular and the relationship between fibroblasts and collagen network did not provide evidence for a preferential histoarchitectural pattern. Vascular structures were generally numerous and arranged in layers. The presence of some layers of granular cells was also observed, as was the presence of partly or fully keratinized layered elements. These features were the expression of a completed epidermal maturation process.
- **Repair process in the presence of N-carboxybutyl chitosan.**
  Ultrastructural analysis identified fibroblasts of elongated shape arranged according to precisely oriented, parallel lines. Vascular structures were

largely present while the inflammatory cellular component was occasional. The collagen network, though rather loose, showed a regular distribution. The general aspect of the derma gave an overall impression of histoarchitectural order which was more evident than in controls. The epidermis exhibited in general a linear junction with the derma, without marked offshots. Overall, the epithelium was organised and cytologically normal, even though the malpighian layer appeared less extended than in controls, as did the whole multilayer of the epidermis. The skin reached its final differentiation with superficial features of keratosis with both types of dressing. After 1 month, more features of the maturation process were identified at morphological analysis (Fig 1).

Prospects

The early steps of skin-tissue repair are sustained by fibroblast proliferation, collagen deposition, angiogenesis and subsequent epithelisation.
In this phase, N-carboxybutyl chitosan gel favours the formation of a loose connective tissue rather than large and dense fibre bundles, facilitating the diffusion of factors and substances and cell proliferation. Then, in the later stages of the normal process of wound healing, when collagen synthesis declines and high oxygen tension is no longer required, many new vascular channels regress; the wound becomes usually avascular and undergoes a transformation into a scar with impaired tissue elasticity. Regression of angiogenesis takes place as soon as chitosan is no longer administred or has been adsorbed; nevertheless, the resulting connective tissue is regularly and orderly structured, without noticeable scars and is endowed with good functionality, i.e. tensile strength. In fact, one of the functions exerted by N-carboxybutyl chi-

Fig. 1. Regenereted skin by chitosan gel: note the evident keratinocytes differentiation even if lipid barrier reconstitution is poor (TEM, 20000x).

tosan appears to be the limitation of the process of wound contraction due to the production of a loose collagen network and the inhibition of large-bundle formation, a point of similarity with heparin and the ability to keep high hydration conditions, a point of similarity with glycosaminoglycans (6, 7).
As far as re-epithelisation is concerned, the fibrin clot acts as a scaffold for migrating epithelial cells. Epithelial cell migration takes place through the fibrous stromal proteins, and epithelial cells secrete collagenase to pass through that stromal medium. For wound healing to take place, a tridimensional supporting lattice is very important; N-carboxybutyl chitosan has been reported to favour rapid re-epithelialisation.
As regards ceramides, several experimental approaches suggest an important role for ceramides in regulating such diverse biological responses as cell-cycle arrest, apoptosis, and cell senescence. *In vitro*, ceramide activates a serine threonine protein phosphatase, and in cells it regulates protein phosphorylation as well as multiple downstream targets (such as interleukin-converting enzyme (ICE)-like proteases, stress-activated protein kinases, and the retinoblastoma gene product) that mediate its distinct cellular effects. This spectrum of inducers of ceramide accumulation and the nature of ceramide-mediated responses suggest that ceramide is a key component of intracellular stress-response pathways (8).
The assessment of barrier function in aged epidermis under basal conditions is known to be misleading, since both barrier integrity and barrier repair are markedly abnormal. As mentioned above, these functional changes can be attributed to a global deficiency in stratum-corneum lipids, resulting in thinning lamellar bilayers in stratum-corneum interstices. The relationship between higher ceramide skin content and improvement in skin hydratation evidences the important role of ceramides in the maintenance of a physiological barrier function of the skin by controlling its hydratation status and counteracting stressing stimuli (9). With a view to attaining optimum cutaneous repair, also the superficial lipid layer should thus be completely reconstituted, especially in elderly subjects where a decrease in stratum-corneum lipids is a major etiological factor for atopic dry skin and a primary event in the transformation into aged dry skin (10). We believe that this goal will be achieved by combining chitosans and ceramides, even though the experience of other researchers indicates that a series of problems - connected mainly with the adsorption of ceramides - remain unresolved.

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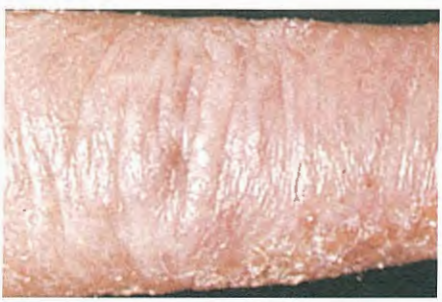

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